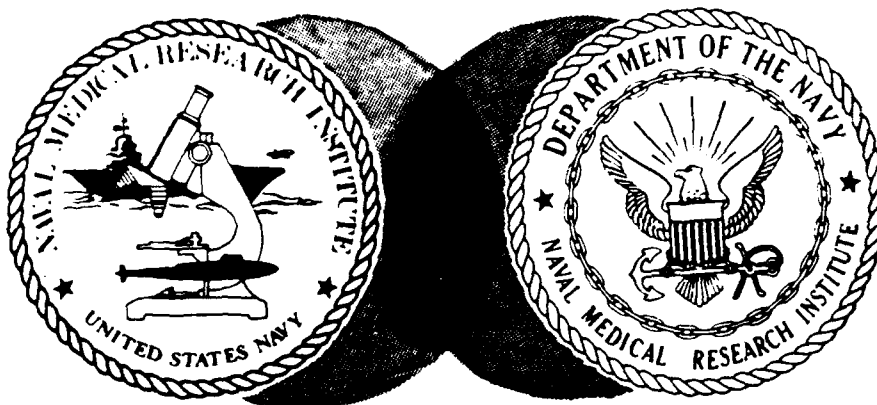


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PREVENTION OF ENDOTOXIN-INDUCED
PULMONARY HYPERTENSION IN PRIMATES
BY THE USE OF A SELECTIVE THROMBOXANE
SYNTHETASE INHIBITOR, OXY 1531

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M.I. ZMUDKA AND P.W. RAINWELL

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- 1) OKY 1581 is an effective Tx synthetase inhibitor in vivo; 2) endotoxin-induced pulmonary hypertension is mediated largely by increased Tx; and 3) the inhibition of Tx synthetase results in shunting of endoperoxides into the prostacyclin pathway.

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Prevention of Endotoxin-Induced Pulmonary Hypertension in Primates by the Use of a Selective Thromboxane Synthetase Inhibitor, OXY 1581^{1, 2}

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ABSTRACT

Endotoxin-induced pulmonary hypertension can be attenuated by nonsteroidal anti-inflammatory drugs and is associated with increased plasma levels of thromboxane (Tx) B₂, prostaglandin (PG) F₂, PGE and PGI₂. Because nonsteroidal anti-inflammatory drugs block prostacyclin production and may also shift arachidonic acid into the lipoxygenase pathway, we have evaluated a selective Tx synthetase inhibitor (OKY 1581) as a means for preventing endotoxin-induced pulmonary hypertension. An LD₅₀ dose of *Escherichia coli* endotoxin (6 mg/kg) was given i.v. to two groups of unanesthetized baboons. Group I received endotoxin alone and Group II was pretreated with i.v. OKY 1581 (2 mg/kg) 10 min before the endotoxin. OKY 1581 produced a significant decrease in the basal plasma TxB₂ from

0.432 ± 0.82 to 0.147 ± 0.032 ng/ml (P < .01), but no significant change in plasma 6-keto PGF_{1α}. After the administration of the endotoxin, Group I developed pulmonary hypertension (from 11 ± 1 to 19 ± 2 mm Hg, P < .005) and an 8-fold increase in plasma TxB₂ (P < .02), whereas Group II did not develop pulmonary hypertension or an increase in plasma TxB₂. However, Group II had a 26-fold increase in plasma 6-keto PGF_{1α} (P < .05). From these studies, we conclude that: 1) OKY 1581 is an effective Tx synthetase inhibitor *in vivo*; 2) endotoxin-induced pulmonary hypertension is mediated largely by increased Tx; and 3) the inhibition of Tx synthetase results in shunting of endoperoxides into the prostacyclin pathway.

The first clinical recognition of septic shock occurred in 1941 when Ebert and Stead described eight cases of circulatory failure during acute infectious diseases. Although Delaunay *et al.* (1947, 1948, 1949) described many of the early vascular effects of endotoxin, it was not until 1955 that it became recognized that endotoxin could be the cause of shock (Weil *et al.*, 1955; MacLean and Weil, 1956). In these studies, it was demonstrated that i.v. endotoxins from a variety of Gram-negative organisms caused a precipitous fall in systemic arterial blood pressure and a simultaneous increase in portal venous pressure. The pulmonary effects of endotoxin were recognized by Thomas (1954) who described rapid labored breathing and by Burrows (1951) who found foci of edema, congestion and

hemorrhage in the lungs of animals given endotoxin. The first systematic evaluation of the pulmonary effects of endotoxin was conducted by Kuida *et al.* (1958) in which endotoxin (5 mg) administered i.v. into a dog caused an increase in the pulmonary arterial pressure, thereby suggesting an increase in pulmonary vascular resistance. Subsequently, endotoxin-induced pulmonary hypertension has been described in the cat (Kuida *et al.*, 1961), sheep (Halmagyi *et al.*, 1963), rhesus monkey (Kuida *et al.*, 1961), baboon (Fletcher *et al.*, 1976), rabbit (Kuida *et al.*, 1961), horse and calf (Tikoff *et al.*, 1966).

A large number of vasoactive agents are released after the i.v. administration of endotoxin. These include histamine (Weil and Spink, 1957; Hinshaw *et al.*, 1961; Hinshaw, 1964), 5-hydroxytryptamine (Armin and Grant, 1957; Davies *et al.*, 1959), angiotensin (Hall and Hodges, 1971), epinephrine and norepinephrine (Nykiel and Glaviano, 1961; Hokfelt *et al.*, 1962; Hall and Hodges, 1971), C_{5a} (Hammerschmidt *et al.*, 1980), endorphins (Holaday and Faden, 1978) and PGs (Kessler *et al.*, 1973; Anderson *et al.*, 1972; Fletcher *et al.*, 1976, 1981). Aspirin, meclofenamate, polyphoretin phosphate and indomethacin all attenuate endotoxin-induced pulmonary hypertension (Hinshaw *et al.*, 1967; Parratt and Sturgess, 1974, 1977; Reeves *et al.*, 1972; Fletcher and Ramwell, 1977). Inasmuch as these drugs

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² The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large. The experiments reported herein were conducted according to the principles set forth in "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication No. (National Institutes of Health) 74-23.

ABBREVIATIONS: PG, prostaglandin; Tx, thromboxane.

are known to inhibit PG formation by blocking cyclooxygenase (Vane, 1971), it strongly suggests that endotoxin-induced pulmonary hypertension is mediated by PGs.

PGs are lipid autocooids derived from polyunsaturated fatty acid precursors by enzymatic cyclooxygenation. Some derivatives from these pathways, $\text{PGF}_{2\alpha}$, PGH_2 , TxA_2 , PGD_2 , LTC_4 and LTD_4 can produce pulmonary hypertension (Kadowitz *et al.*, 1981; Schiantarelli *et al.*, 1981; Altura and Chand, 1981). PGE_2 , $\text{PGF}_{2\alpha}$ and TxB_2 are all elevated after the administration of endotoxin and the time course of their presence in plasma corresponds to the development of pulmonary hypertension (Anderson *et al.*, 1975; Parratt and Sturgess, 1975, 1977; Fletcher *et al.*, 1976, 1981; Fletcher and Ramwell, 1977; Harris *et al.*, 1980). Because nonsteroidal anti-inflammatory drugs act by inhibiting cyclooxygenase, they can cause shunting of arachidonic acid into the lipoxygenase pathway (Samuelsson, 1980). This could be detrimental as the leukotrienes are thought to cause increased vascular permeability (Ueno *et al.*, 1981; Seeger *et al.*, 1981). In addition, nonsteroidal anti-inflammatory drugs also inhibit prostacyclin production (MacIntyre *et al.*, 1978). Prostacyclin is a potent inhibitor of platelet aggregation (Moncada *et al.*, 1976) and white cell adhesion (Boxer *et al.*, 1980; McGillen *et al.*, 1980). Infusions of prostacyclin are beneficial in endotoxin shock (Fletcher and Ramwell, 1980; Krausz *et al.*, 1981; Lefer *et al.*, 1980), cerebral vascular ischemia (Hallenbeck and Furlow, 1979), myocardial ischemia (Araki and Lefer, 1980), aspiration pneumonia (Utsunomiya *et al.*, 1981) and peripheral vascular disease (Belch *et al.*, 1981; Hossman *et al.*, 1981). Thus, the inhibition of prostacyclin production by the cyclooxygenase inhibitors may not be advantageous. With the recent availability of a selective inhibitor of Tx synthetase, its use might provide a better approach because it would not increase the lipoxygenase products, but would increase prostacyclin as well as the other classical PGs (Miyamoto *et al.*, 1980; Feuerstein and Ramwell, 1981a).

The purpose of this study is to evaluate the ability of a selective Tx synthetase inhibitor to prevent endotoxin-induced pulmonary hypertension in subhuman primates.

Methods

Adult male baboons (*Papio anubis*) weighing 30 to 40 kg were anesthetized with ketamine (15 mg/kg) and, utilizing aseptic technique, arterial and pulmonary artery catheters (Edwards Laboratory, Santa Anna, CA) were inserted through a femoral cut-down. The baboon was then placed in a restraining chair designed to maintain an upright posture and allowed to recover from the anesthesia. No additional anesthesia was given until the end of the study when the catheters were to be removed. Cardiac outputs were measured by the thermal dilution method and calculated on a computer program for a PDP-12 computer (Digital Equipment Corporation, Marlboro, MA). Systemic and pulmonary artery pressures were measured with transducers (Gould-Statham model P23) and recorded on an eight-channel recorder (Gould-Brush). Heart rate was determined from the pressure tracings.

Two groups of animals were studied: Group I (control, $n = 5$) received endotoxin alone (Difco Laboratories, Detroit, MI; *Escherichia coli*: 0111:B4, 6 mg/kg) and Group II ($n = 6$) received a bolus of 2 mg/kg of OKY 1581 (fig. 1) [sodium-(E)-3-[4-(3-pyridylmethyl)phenyl]-2-methylacrylate] i.v. 10 min before the endotoxin. This dose of OKY 1581 is twice the dose necessary to convert a 100% arachidonic acid-induced mortality (4 mg/kg) in rabbits into 100% survival (Miyamoto *et al.*, 1980). An LD_{50} dose (6 mg/kg) of *E. coli* endotoxin was given to each animal. The animals were allowed to stabilize for at least 1 hr before obtaining the base-line measurements. All variables were measured just before giving the endotoxin and then 5, 15, 30, 60 and 120 min after the

i.v. administration of the endotoxin. At the conclusion of the study, the baboons were anesthetized with ketamine (15 mg/kg), the catheters were removed and the animals were returned to their cage and observed for survival. Survival was arbitrarily defined as living longer than 7 days. All animals that were alive after 7 days were also alive after 1 month.

Platelet and white blood cell counts were determined by an electronic particle counter (Coulter, model ZBI). Arterial and mixed venous blood gases were measured on a blood gas analyzer (Instrumentation Laboratories, Lexington, MA, model 813). Blood for PG assay was collected in iced tubes containing EDTA (1.5 mg/ml) and indomethacin (10 $\mu\text{g}/\text{ml}$), immediately centrifuged at 4°C (2500 rpm for 15 min, International PR-6000 centrifuge) and the plasma frozen (-70°C) until assayed. Stable end products of TxA_2 (TxB_2) and prostacyclin (6-keto $\text{PGF}_{1\alpha}$) were measured by radioimmunoassay of the unextracted plasma (Morris *et al.*, 1981). The antibody for 6-keto $\text{PGF}_{1\alpha}$ is prepared in this laboratory and the cross-reactivity with other PGs is: TxB_2 (1%), PGE_2 (10%) and $\text{PGF}_{2\alpha}$ (7%). Antibody for TxB_2 was kindly given to us by Dr. L. Levine (Brandeis University, Boston, MA) and demonstrated a cross-reactivity of less than 2% with $\text{PGF}_{2\alpha}$, PGE_2 and 6-keto $\text{PGF}_{1\alpha}$. The coefficient of variation for duplicate samples was less than 10%.

Data analysis. The results are expressed as means \pm S.E.M. Student's *t* test for unpaired samples was used for comparing data between Groups I and II and the paired *t* test for evaluating differences within a group. A *P* value of less than .05 was considered significant.

Results

The basal levels of TxB_2 and 6-keto $\text{PGF}_{1\alpha}$, respectively, were 0.432 ± 0.082 and 0.067 ± 0.023 ng/ml. The administration of 2 mg/kg of OKY 1581 alone produced a significant decrease in basal plasma TxB_2 to 0.147 ± 0.032 ng/ml ($P < .01$), but no significant change in the basal plasma 6-keto $\text{PGF}_{1\alpha}$ (0.085 ± 0.046 ng/ml, $P > .05$).

Table 1 contains the hemodynamic data for Groups I and II. There was no change in the base-line values after the administration of either saline (Group I) or 2 mg/kg of OKY 1581 (Group II). Thus, the base-line values listed in table 1 represent

Chemical name: Sodium-(E)-3-[4-(3-pyridylmethyl)phenyl]-2-methylacrylate

Structural Formula:

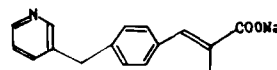


Fig. 1. Structure of OKY 1581.

TABLE I
Hemodynamic effects of endotoxin in baboons*

Parameter	Group I ^b		Group II ^c	
	Base line	5 min	Base line	5 min
MAP ^d	115 \pm 3	101 \pm 6*	105 \pm 5	85 \pm 12
PAP	11 \pm 1	19 \pm 2***	10 \pm 2	11 \pm 2***
CO	3.3 \pm 0.2	2.6 \pm 0.2	3.5 \pm 0.5	2.4 \pm 0.5
HR	123 \pm 4	147 \pm 2**	119 \pm 4	147 \pm 4***

* Results are expressed as mean \pm S.E.M.

^b $N = 5$, base-line values were obtained 10 min postsaline. Immediately after the base lines were measured, 6 mg/kg of *E. coli* endotoxin was injected i.v.

^c $N = 6$, pretreated with OKY 1581 (2 mg/kg) 10 min before the base-line parameters. Immediately after the base lines were measured, 6 mg/kg of *E. coli* endotoxin was injected i.v.

^d MAP, mean arterial pressure (millimeter of mercury); PAP, mean pulmonary artery pressure (millimeter of mercury); CO, cardiac output (liters per minute); HR, heart rate (beats per minute).

* $P < .05$; ** $P < .02$; *** $P < .005$ comparing base line to 5 min; *** $P < .05$ comparing Group I with Group II.

those values obtained after the administration of either saline or OKY 1581, but just before the administration of the endotoxin. Five minutes after the endotoxin, there was a significant decrease in mean arterial pressure ($P < .05$) in Group I. Group II also had a decrease in mean arterial pressure, however, there was greater animal variability. Two of the animals in Group II had a 60 mm Hg decrease in mean arterial pressure, one had a 20 mm Hg increase and the remainder of the animals had about a 20 mm Hg decrease.

There was a 2-fold increase in pulmonary artery pressure in Group I ($P < .005$), but no significant increase in Group II. Both groups had nonsignificant decreases in cardiac output and significant increases in heart rate (Group I, $P < .02$; Group II, $P < .005$).

The pulmonary hypertension in Group I corresponded to an 8-fold increase in plasma TxB₂ ($P < .02$). There was a small but

nonsignificant increase in plasma TxB₂ in Group II (fig. 2). In Group I, there was a progressive increase in plasma 6-keto PGF_{1 α} ($P < .01$), whereas in Group II, there was an early 26-fold increase ($P < .05$) which corresponded to the increase in TxB₂ seen in Group I (fig. 3).

Although both groups developed a significant neutropenia (Group I: 40 ± 7 , Group II: $33 \pm 8\%$ of base line, $P < .02$) and a decrease in platelet count [Group I: 277 ± 86 – $184 \pm 53 \times 10^3/\text{mm}^3$, ($P < .02$) and Group II: 282 ± 97 – $192 \pm 64 \times 10^3/\text{mm}^3$, ($P < .02$)], there were no significant differences between the two groups.

There was a significant decrease in PaO₂ from 93 ± 2 to 77 ± 4 mm Hg ($P < .005$) in Group I and a nonsignificant decrease in Group II (92 ± 2 – 82 ± 5 mm Hg, $P < .05$).

There was no significant difference in survival between the two groups: Group I (2 of 5) and Group II (2 of 6).

Fig. 2. Plasma TxB₂ levels during baboon endotoxin shock. TxB₂ is the stable product of TxA₂. The control group (Group I) was pretreated with saline and Group II received 2 mg/kg of OKY 1581 10 min before the base-line measurements. Both groups received 6 mg/kg of *E. coli* endotoxin immediately after the base-line. The values represent the mean \pm S.E.M. with $n = 5$ in Group I and $n = 6$ in Group II.

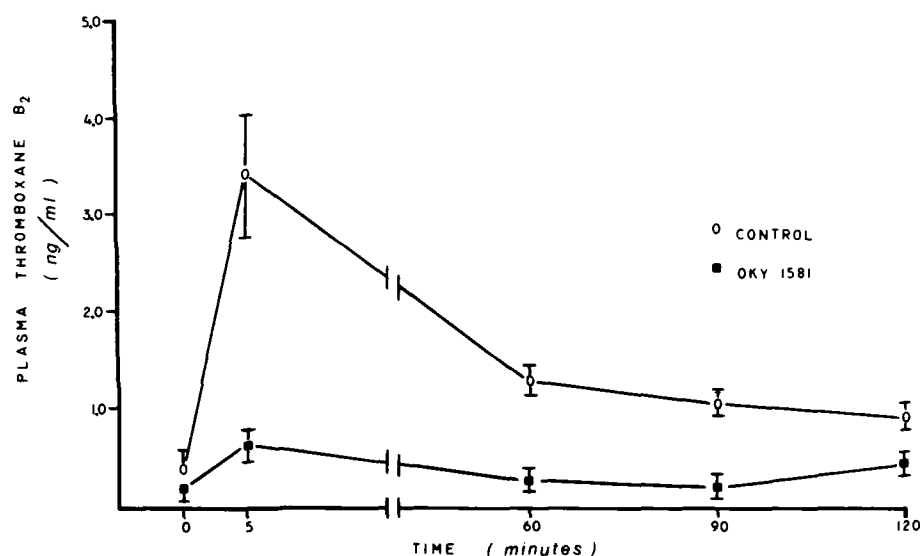
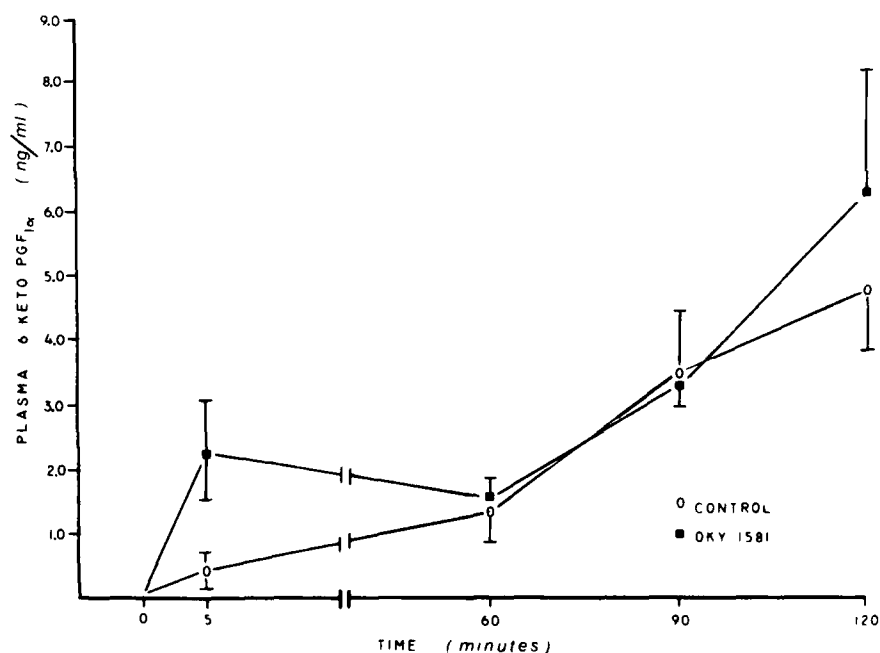


Fig. 3. Plasma 6-keto PGF_{1 α} levels during baboon endotoxin shock. 6-keto PGF_{1 α} is a stable product of prostacyclin. Both groups are the same as in figure 2.



Discussion

The present study demonstrates for the first time in primates that: 1) OKY 1581 is an effective Tx synthetase inhibitor under conditions of pathologic TxA_2 production; 2) the specific inhibition of Tx production prevents endotoxin-induced pulmonary hypertension; and 3) the inhibition of Tx synthetase with OKY 1581 results in a shift of the endoperoxides into the prostacyclin pathway.

The possible mechanisms by which endotoxin might produce an increase in pulmonary artery pressure include: 1) a direct effect of endotoxin on smooth muscle contraction, 2) cellular obstruction of the pulmonary microvasculature, or 3) endotoxin-induced release of smooth muscle-contracting substances. It is unlikely that endotoxin has a direct effect on the pulmonary vasculature or on pulmonary parenchymal cells because *in vitro* the pulmonary effects of endotoxin are dependent upon the perfusate composition (Hinshaw *et al.*, 1957; Kux *et al.*, 1972). The incubation of parenchymal lung strips with endotoxin *in vitro* results in suppression of PG production, whereas when endotoxin is given *in vivo*, the lung strips have increased PG production (Feuerstein and Ramwell, 1981b). Similar results, both *in vivo* and *in vitro*, have been found with the rat aorta (Pomerantz *et al.*, 1980). Because i.v. endotoxin produces both leukocyte and platelet sequestration in the lung (Coalson *et al.*, 1970; Stein *et al.*, 1967) and cinephotomicrography has shown showers of pulmonary microemboli (Robb *et al.*, 1972), it is possible that the early increase in pulmonary vascular resistance might be attributable to mechanical occlusion of the pulmonary vessels (Pennington *et al.*, 1973). However, using an isolated lung perfused with acid-citrate-dextrose blood, there was leukocyte sequestration, but no increase in perfusion pressure. In addition, pulmonary hypertension still occurred when perfusion was done with neutropenic and thrombocytopenic heparinized blood (Kux *et al.*, 1972). *In vivo*, using either low-dose endotoxin (15 $\mu\text{g}/\text{kg}$) (Hales *et al.*, 1981), indomethacin pretreatment (Fletcher and Ramwell, 1978) or Tx synthetase inhibition (OKY 1581), there is still neutropenia and thrombocytopenia but no pulmonary hypertension. These studies suggest that the increase in pulmonary vascular resistance seen after the injection of endotoxin is not due to mechanical obstruction of the vascular bed or a direct effect of endotoxin, but may be related to the *in vivo* release of humoral factors.

Previously, it was shown that endotoxin-induced pulmonary hypertension corresponds to elevations in plasma TxB_2 and $\text{PGF}_{2\alpha}$, both of which produce pulmonary hypertension (Fletcher *et al.*, 1976, 1981). In addition, endoperoxide analogs are also potent pulmonary vasoconstrictors (Bowers *et al.*, 1979) and thus the endoperoxides must also be considered as possible mediators. The pulmonary hypertension, thrombocytopenia, and neutropenia seen after endotoxin administration are also seen after i.v. platelet activating factor (McManus *et al.*, 1981). Based on the results of the present study which used a specific Tx synthetase inhibitor, we now suggest that endotoxin-induced pulmonary hypertension is mediated predominately by increased Tx production.

The source of Tx cannot be determined because many cells are capable of producing Tx under both basal and pathological conditions. There is some information from the literature to suggest that the lung is the major site of Tx production. After i.v. endotoxin in a rat, the incubation of lung parenchymal strips show increased Tx production (Feuerstein and Ramwell, 1981b). There is also increased Tx levels in the pulmonary

lymph of sheep (Frolich *et al.*, 1980). Although these studies suggest that the lung may be a site of Tx production, they provide no insight as to whether it is from the lung itself or from the trapped formed elements. The *in vitro* incubation of lung tissue with endotoxin results in decreased PG production (Feuerstein and Ramwell, 1981b). The *in vitro* incubation of white blood cells with endotoxin causes Tx production (Spagnuolo *et al.*, 1980). Thus, leukocytes are a more likely source of Tx, although neutropenic animals still develop pulmonary hypertension (Pengleton *et al.*, 1975). The interaction of platelets with endotoxin is complicated and certainly species-dependent (Morrison and Ulevitch, 1978). In general, endotoxin does not stimulate platelet Tx production (Fletcher and Ramwell, 1980). The observation that the platelet count decreased despite increased levels of prostacyclin is consistent with our previous findings that prostacyclin was ineffective in inhibiting endotoxin-induced platelet aggregation *in vitro* and that the pretreatment of dogs with prostacyclin did not prevent the decrease in platelet count (Fletcher and Ramwell, 1980).

The present study is an extension of previous studies that have used either cyclooxygenase inhibitors or nonselective Tx synthetase inhibitors (such as imidazole) during endotoxin shock. In a preliminary report, imidazole has been used to prevent endotoxin-induced pulmonary hypertension and Tx production in sheep (Huttemeier *et al.*, 1981). However, the selectivity and efficacy of imidazole appears to be dependent upon the species and the method used to stimulate Tx production because imidazole is not able to block Tx production in either cat or primate endotoxin shock or in rat renal microsomes (Smith *et al.*, 1980b; Casey *et al.*, 1981; Strand *et al.*, 1981). On the other hand, imidazole is effective in preventing platelet Tx production *in vitro* (Moncada *et al.*, 1977), endotoxin-induced *in vivo* Tx production in rats and sheep (Frolich *et al.*, 1980; Cook *et al.*, 1980) and during myocardial ischemia in cats (Smith *et al.*, 1980a). The explanation for these differences remains unclear. OKY 1581 is a specific Tx synthetase inhibitor (Miyamoto *et al.*, 1980; Feuerstein and Ramwell, 1981a) and thus this study provides the first definitive implication of Tx as the primary mediator of endotoxin-induced pulmonary hypertension in subhuman primates.

In this study, the hemodynamic effects of endotoxin alone (Group I) are consistent with previous studies (Fletcher *et al.*, 1976, 1981). The fall in cardiac output after endotoxin administration is generally thought to be related to the acute pulmonary hypertension causing a decrease in left ventricular filling pressure (Traber *et al.*, 1981). The animals in Group II did not develop pulmonary hypertension, but still had a decrease in mean arterial pressure without a significant decrease in left ventricular filling pressure. These findings suggest that the decrease in cardiac output and mean arterial pressure is not solely related to the acute pulmonary hypertension. Prostacyclin is a potent vasodilator and its infusion into awake subhuman primates results in systemic hypotension, a decrease in cardiac output and a variable response in heart rate (Fletcher and Ramwell, 1979; Hintze *et al.*, 1978). The hemodynamic changes seen in this study may be attributed to the increased plasma prostacyclin levels. It is possible that the blunted pulmonary pressor response to the endotoxin in Group II is a result of the increased prostacyclin production. The pretreatment of dogs with prostacyclin did not block the pulmonary hypertension in response to endotoxin (Fletcher and Ramwell, 1980). The amount of prostacyclin infused (20 ng/kg/min) may have been sufficient to raise the plasma 6-keto $\text{PGF}_{1\alpha}$ levels to the

extent observed in Group II, however, plasma levels from that study are not available. Thus, it is possible that the lack of pulmonary hypertension in Group II is not just related to the inhibition of thromboxane formation, but may be dependent upon an increase in the ratio of prostacyclin to Tx.

Our observation that a Tx synthetase inhibitor results in increased amounts of prostacyclin is consistent with previous studies both *in vivo* and *in vitro* (Nijkamp *et al.*, 1977; Vermynen *et al.*, 1981). The mechanism for this could result from a shunting of the endoperoxides from the Tx pathway into both the classical PG and prostacyclin pathways. We did not measure plasma PGF_{2α} or PGE and thus can only speculate that there might have been an increase in these classical PGs as well as in the prostacyclin pathway. An imidazole derivative, UK 37248 [4-(2-1H-imidazole-1yl)ethoxyl benzoic acid], given to humans, decreased basal plasma TxB₂ and increased plasma 6-keto PGF_{1α} (Vermynen *et al.*, 1981), which is consistent with our findings with OKY 1581. Recently, OKY 1581 was reported to be ineffective in decreasing basal Tx in baboons (Ashim *et al.*, 1981). The difference between that study and our study may be related to the fact that we used a higher dose (2 mg/kg) and we administered it as a bolus rather than as a continuous infusion. The duration of Tx synthetase inhibition by OKY 1581 was not determined in our study.

The development of pulmonary hypertension during sepsis carries a poor prognosis (Vito *et al.*, 1974; Clowes *et al.*, 1970). Based upon the observations in this study, it may be possible to prevent or attenuate the development of septic-induced pulmonary hypertension by the use of selective Tx synthetase inhibitors. The use of OKY 1581 did not improve survival in our study, therefore its effects on the prognosis of the septic patient remains to be determined. Likewise, Tx has been implicated in a variety of other pathologic conditions such as: 1) shock; 2) myocardial ischemia; 3) cerebral vascular insufficiency; 4) deep vein thrombosis; and 5) pulmonary embolism. The use of selective Tx synthetase inhibitors in these disease entities may not only provide valuable information concerning the pathophysiology but may also improve morbidity and mortality.

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